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Divide and Die: Cell Cycle Events as Triggers of Nerve Cell Death

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For over a decade, evidence has mounted that nerve cell death in the CNS is often intimately linked to a process of cell division. Mitotic markers appear in neurons at risk for death in a variety of neurodegenerative conditions, in mouse and in humans. Beyond correlation, studies have shown that experimentally driving the cell cycle in a mature neuron leads to cell death rather than cell division, and blocking cell-cycle initiation can prevent many types of neuronal cell death. This unlikely linkage of cell cycle and cell death pathways is little appreciated among neuroscientists. As only one example, bromodeoxyuridine (BrdU) labeling is often uncritically accepted as proof of neurogenesis when it may well be attributable to a cell cycle-related cell death. This review is meant to enhance appreciation for the relevance of this phenomenon to development and neurodegenerative diseases, in particular the neurodegeneration found in Alzheimer's disease (AD). A brief overview of the participation of mitotic events in human Alzheimer's disease and its mouse models is presented. Against this background, we consider evidence that links various APP (amyloid precursor protein) binding proteins with the cell cycle in Alzheimer's disease. We also examine the role played by oxidative stress as a trigger for cell cycle-related neuronal death. Finally, we discuss the biochemical details of the lethal neuronal cell cycle events and present evidence that non-canonical pathways of DNA replication are probably involved.

Background

Since the earliest days of neurobiology, the study of neuronal cell death has been a field full of surprises. For example, the very contention that the death of neurons might be a normal part of the developmental program of the brain was greeted initially with great skepticism, and its formal demonstration by Hamburger (1975) and others came as quite a surprise to most workers in the

field. Later, after the existence of target-related cell death and its dependence on trophic support had been firmly established, Oppenheim and colleagues uncovered yet another surprise. They showed that blocking neuronal activity during the critical period of a neuron, rather than mimicking the effects of target deprivation as expected, actually spared the doomed neurons from the fate of death (Pittman and Oppenheim, 1979). It is in this long tradition of surprises that we review a series of discoveries relating instances of neuronal cell death to a process normally linked to birth and regeneration: the cell cycle. By this time, however, the existence of a paradox such as this should come as no surprise.

The textbooks are clear: after they leave the ventricular zone (VZ) or subventricular zone (SVZ), neurons are aggressively postmitotic. The nature of this permanent exit from the cell cycle is still poorly understood, but the fact that no known cancer originates from an adult neuron is strong evidence that the restriction knows absolutely no work around. It is true that a small number of neurons are generated in the adult, but their origin is believed to be in the remnants of the VZ and SVZ (and their final fate is still debated).

The strange case of the T-antigen transgenics

The fact that a differentiated neuron never divides is an enormous impediment to tissue culture studies of nerve cells. Primary cultures of neurons cannot be propagated; they must be continually reestablished (with apologies to aficionados of neurosphere culture). In 1992, Feddersen et al. (1992) decided that they would rectify this situation using genetic engineering of transgenic mice. They were determined to force cell division in a neuron, so they took a powerful oncogene, the SV40 (simian virus 40) T-antigen, and drove its expression in transgenic mice using a Purkinje cell-specific promoter from the *pcp2* gene. Legend has it they were eagerly awaiting culture dishes full of dividing Purkinje cells; what they got instead was ataxic mice. The founder transgenics did indeed express T-antigen exclusively in Purkinje cells and, through its sequestration of the retinoblastoma protein, released what appeared to be an initial attempt at a cell cycle. Purkinje cell nuclei incorporated BrdU and swelled in size, but rather than dividing, the Purkinje cells died. In this same year, al-Ubaidi et al. (1992) attempted an identical feat with a rhodopsin promoter and achieved the same surprising results: blind mice attributable to dead photoreceptors. Other authors were investigating the effect of elimination of the entire *retinoblastoma* gene. Three dif-

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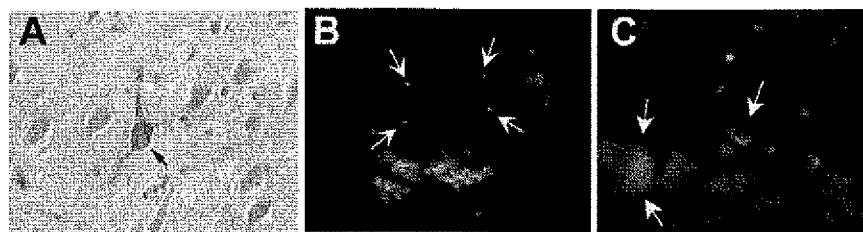


Figure 1. Cell cycle events in hippocampal pyramidal neurons of human and mouse. *A*, A neuron in the CA1 hippocampal region of a patient who died with Alzheimer's disease. The neuron indicated by the black arrow is immunopositive for the PCNA protein, an S-phase marker (from Busser et al., 1998). *B*, A CA3 area pyramidal cell from an Alzheimer's patient hybridized with a unique genomic probe from chromosome 11. Note the four spots of hybridization (white arrows) indicating the full replication of this portion of the genome (from Yang et al., 2001). *C*, The CA1 region of an R1.40 transgenic mouse stained with an antibody to cyclin A (green) and counterstained with propidium iodide (red). Note that the three neurons are immunopositive for this S-phase marker (Y. Yang, B. T. Lamb, and K. Herrup, unpublished observations).

ferent laboratories reported essentially the same phenotype: in the absence of RB, neurons exiting the ventricular zone continue to proliferate, fail to differentiate, and subsequently die (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). From these early experiments, the concept arose that a cell division forced on a mature neuron is a lethal event.

Over the next several years, a body of correlative data began to accumulate to further develop this theme and move it from the realm of transgenic oddity into the mainstream of the neurodegeneration literature. Herrup and Busser showed that two models of CNS target-related cell death were accompanied by the reexpression of cell cycle proteins and incorporation of BrdU into the DNA of the target-deprived neurons hours before their death (Herrup and Busser, 1995). Tissue culture studies from the laboratories of Greene, Park, and others provided more detailed mechanistic data on the process. These groups were also the first to show that blocking the cell cycle with cyclin-dependent kinase inhibition could block neuronal cell death in culture (Park et al., 1996, 1997). Finally, a substantial number of laboratories used immunocytochemistry to show the ectopic neuronal reexpression of cell cycle proteins in regions of human brain in which degeneration occurs. Alzheimer's disease is reviewed in greater detail below, but cell cycle-associated neurodegeneration is also found in amyotrophic lateral sclerosis, stroke, and some forms of encephalitis (Jordan-Sciutto et al., 2002; Love, 2003; Ranganathan and Bowser, 2003). The fact that cell cycle events are bedfellows, however strange, of neuronal cell death in many human neurodegenerative diseases compels us to pose several difficult but important questions.

The questions

The prevalence of cell cycle events in the process of neuronal cell death means that we are facing a challenge of some magnitude. Clearly the therapeutic dimension of the discoveries urges thorough attention. However, how does one study cell cycle in a cell that by definition does not divide? We have addressed a series of questions in this review in an effort to put limits on the extent of our current ignorance. First, we consider the phenomenology of cell cycle reactivation in the context of a single human disease, Alzheimer's disease, and its mouse models. This normative work serves as a platform to ask more mechanistic questions. What kind of events can trigger a lethal mitosis in a neuron? What is the nature of the cellular signaling events involved? Just what is a cell cycle in an adult neuron, and what is the relationship of the chemistry of this attempt at rejuvenation to that seen in a population of normally dividing cells? We close with a set of predictions for the future of this exciting field.

A statement of the problem: cell cycle events in Alzheimer's disease

AD is the most common form of late-life dementia. It presents with a range of problems that include the inability to form recent memories, executive dysfunction, and other behavioral symptoms. Instances of AD are divided into two basic types: familial and sporadic. Familial forms (FAD) are relatively rare and are nearly always caused by a single autosomal dominant mutation with near 100% penetrance. They typically strike the victim before the age of 65 and thus are known as early-onset AD. Sporadic AD, in contrast, strikes after 65. Although genetic risk factors are known for sporadic AD, none is of sufficient predictive value to make it a cost-effective screening tool.

On autopsy, the pathological signature of AD is a collection of abnormal deposits: neurofibrillary tangles consisting of aggregates of hyperphosphorylated forms of the microtubule-associated protein, tau, and waxy protein plaques consisting of fragments of the membrane protein APP. Neuronal death is regionally variable with heavy losses of neurons (up to 80%) found in hippocampus and other limbic regions of cortex, as well as in basal nucleus, locus ceruleus, and the dorsal raphe. Although familial forms account for <10% of all AD, much of our knowledge of the biological basis of the disease comes from these forms. Most FAD can be accounted for by mutations in one of three major disease genes: APP itself or the subunits of the APP-cleaving γ -secretases, PS1 (presenilin 1) or PS2. A full articulation of the amyloid hypothesis is well outside the focus of this article (for information and a taste of the ongoing debates in the field, see Neve and Robakis, 1998; Selkoe, 2002; Lee et al., 2004). The puzzle in the field has always been that, although dementia in AD is likely caused by the neuronal atrophy and loss, the path from plaques and tangles to regionally variable cell loss is not clear. What is becoming clear, however, is that the path passes directly through the cell cycle.

Vincent and Davies were perhaps the first to show definitively that active cell cycle components are present in neurons in the AD brain (Vincent et al., 1996). In close succession, several other laboratories reported that a wide range of cell cycle proteins, including cell cycle inhibitors, are present in neurons in autopsy specimens from AD patients (Fig. 1A) but not in age matched controls (Arendt et al., 1996; McShea et al., 1997; Nagy et al., 1997; Busser et al., 1998). These studies all rely primarily on protein-based evidence (e.g., immunocytochemistry) to show the reappearance of cell cycle kinases, their activators, and inhibitors; the data offer only indirect support of the idea that neurons are entering a real cell cycle. Yang et al. (2001) made an important additional observation. The authors used fluorescent *in situ* hybridization (FISH) to show that actual DNA replication has occurred in at-risk neurons in the AD brain (Fig. 1B). The same laboratory also showed that the involvement of cell cycle processes occurs in early stages of the disease process, not just during end-stage disease (Y. Yang et al., 2003). In both immunocytochemical and FISH preparations, 4–10% of the neurons in susceptible regions are positive for cell cycle evidence, whereas in a disease with a 10 year course, <0.05% would be expected if death after cycling were a rapid process. To account for the relatively high percentage of cell cycle-positive neurons, Yang et al. specu-

lated that the neurons in the AD brain must take a long time to die after they begin their “cycle.”

When one is dealing with postmortem human brain tissue as source of material, one has little “experimental” control. DNA replication, easily shown with BrdU incorporation in mouse, must be shown by other means in humans, and although longitudinal pathological studies may be straightforward in mice (in which genotype and environmental variation can be held to a minimum) in humans, nearly all pathological tissue is from individuals at the end of the disease course. The ability to examine earlier stages depends heavily on good fortune. Thus, there was strong impetus to develop a number of different transgenic mouse models of AD that overexpress the human FAD genes in the context of the mouse (Hock and Lamb, 2001). Plaques are found in many of these models, especially when APP and PS1 transgenes are combined, but none develops any neurofibrillary tangles. In a recent example of a triple transgenic mouse (APP/PS1/tau), tangles were observed in the neuropil, documenting that such deposits are possible in mice (Oddo et al., 2003). This would seem to be a good start on mimicking the neuropathology of AD, yet the neurons just do not seem to get the message that there is a problem. Neuritic atrophy is found in some transgenics (German et al., 2003), but only one of the nearly one dozen mouse models has any reported loss of neurons (Calhoun et al., 1998). Furthermore, in this line, the loss amounts to less than one-quarter of the hippocampal CA1 pyramidal neurons. Other laboratories have searched for cell loss and found none (Irizarry et al., 1997). Abnormal behavioral phenotypes are found, including memory deficits, suggesting that neuronal dysfunction is going on at some level. The source of the dysfunction is unclear, however, because clearance of A β plaque peptide by immunization seems to result in a behavioral “cure” within days, although the plaques themselves linger for weeks (Dodart et al., 2002; Kotilinek et al., 2002).

But what does all this have to do with the cell cycle? The absence of neurodegeneration in model after model in which the other features of the AD pathology are captured so well is a bit odd. The quantification of DNA replication in human AD brain, however, suggests that cycling neurons might take a long time to die, perhaps as much as 1 year (Busser et al., 1998; Yang et al., 2001; Y. Yang et al., 2003). This prompted Yang and Herrup (2004) to determine whether the reexpression of cell cycle markers, the presumed beginning of the neuronal cell loss, were observed in any of the mouse models. It is. In four different plaque-bearing models, both immunocytochemistry and FISH successfully show that neurons in the most vulnerable areas have begun a true cell cycle event (Fig. 1C), although neuronal cell loss has not been reported in any of these models. The timing of these events is also informative. In the best-studied model, cell cycle events are already present by 6 months, many months before the first evidence of plaques at over 1 year.

This view of AD from the vantage point of the cell cycle suggests several conclusions, but also raises many questions. The human studies make it plain that cell cycle events are commonly associated with the death of neurons in the AD brain. Furthermore, their presence in the APP transgenic mice imparts a certain unity to the mouse genetic models and the human pathology that had been missing. However, although the concordance of mouse and man is gratifying in some ways, it is troubling in others. The relative well-being of the “cycling” mouse and human neurons leads to the following question: if a cell cycle is such a bad thing, how is it that many neurons can “cycle” yet not die? We consider this and other questions further below.

The signaling events linking the amyloid precursor protein to cell cycle and death

One of the strongest pieces of evidence in support of the hypothesis that the A β plaque-forming peptide is the true culprit in Alzheimer’s comes from human genetics. The three major FAD genes all encode proteins that enhance the processing of APP into the highly amyloidogenic forms of A β . The logic is compelling, yet, in a truly rigorous sense, the genetics only points to APP and the γ -secretase as disease-causing entities. The A β connection is inferential. This is relevant because of the possibility that APP itself may act as a signaling receptor and influence neuronal survival in different ways. This idea was first proposed on the basis of its predicted amino acid sequence, which suggested that APP was a type 1 intrinsic membrane protein consistent with the structure of a “cell surface receptor” (Kang et al., 1987). That APP might actually function this way was supported when it was found that its intracellular domain interacts with the heterotrimeric G-protein G_o (Nishimoto et al., 1993) and further that a percentage of APP is found on the cell surface in neurons (Jung et al., 1996; Storey et al., 1996; Perez et al., 1997).

As noted above, a failure of regulation of the cell cycle, which can push neurons into apoptotic death, occurs in neurons in AD brain. However, the signaling mechanisms that trigger these events have not been defined. The discovery that at least one of the signaling pathways mediated by APP overexpression leads to neuronal DNA synthesis and apoptosis (Chen et al., 2000; McPhie et al., 2003) suggests that this signaling pathway may play a role in the coexistence of both the cell cycle events and the neurodegeneration seen in AD. A search of cDNA libraries uncovered genes encoding proteins that interact with the cytoplasmic domain of APP. One of these, APP-BP1 (binding protein 1) (Chow et al., 1996), drives the cell cycle through the S-M checkpoint in dividing cells, and overexpression of APP-BP1 in neurons causes them to die (Chen et al., 2000). Notably, expression of FAD mutants of APP in neurons results in (1) an increase in expression of APP-BP1, (2) entry of the neurons into the S-phase of the cell cycle, and (3) neuronal apoptosis. Furthermore, APP-BP1 is overexpressed in at-risk regions of human AD brain relative to cognitively intact controls. APP-BP1 is thus a candidate for participation in the events leading to the loss of cell cycle regulation and DNA replication that appears to occur in AD neurons.

A second binding protein for APP, PAK3 (p21-activated kinase 3), is a serine/threonine kinase (McPhie et al., 2003). This molecule also is implicated in the DNA synthesis and neuronal apoptosis caused by FAD mutants of APP. A dominant-negative kinase mutant of PAK3 inhibits the neuronal apoptosis and DNA synthesis; this effect is abolished by deletion of the PAK3 APP-binding domain or by coexpression of a peptide representing this binding domain. The involvement of PAK3 specifically in FAD APP-mediated apoptosis rather than in general apoptotic pathways is suggested by the facts that a dominant-positive mutant of PAK3 does not alone cause neuronal apoptosis and that the dominant-negative mutant of PAK3 does not inhibit chemically induced apoptosis. Pertussis toxin, which inactivates the heterotrimeric G-proteins G_o and G_i, inhibits the apoptosis and DNA synthesis caused by FAD APP mutants; the apoptosis and DNA synthesis are rescued by coexpression of a pertussis toxin-insensitive G_o. FAD APP-mediated DNA synthesis precedes FAD APP-mediated apoptosis in neurons, and inhibition of neuronal entry into the cell cycle inhibits the apoptosis.

It is hypothesized that APP and its C-terminal binding proteins, G_o PAK3 and APP-BP1, have a regulated interaction that activates a signaling pathway important for normal brain func-

tion, perhaps regulating synaptic remodeling or neurogenesis during learning. Dysfunction of this interaction by FAD mutations in APP causes sustained disruption of the pathway in neurons, leading to entry into the cell cycle and subsequent apoptosis. Thus, this signaling pathway may play a role in AD neurodegeneration.

Oxidative stress, cell cycle, and neurodegeneration

The focus on APP and its metabolites is a strong tradition in the Alzheimer's field. As mentioned above, the logic for this emphasis in understanding FAD is clear. However, >90% of the current cases of AD are believed to be sporadic, in which the largest risk factor by far is age. The biological meaning of age is a difficult concept, one that has seen more and more attention in recent years. Many variables change slowly during the course of an organism's lifetime, but one of the most reliable is the widespread accumulation of oxidative damage. Whereas oxygen is essential for life, its metabolites, known as reactive oxygen species (ROS), can cause extensive cellular damage. Up to 1% of the mitochondrial electron flow can generate ROS, which are detoxified by cellular antioxidants. However, a deleterious condition known as oxidative stress occurs when ROS levels exceed the antioxidant capacity of the cell. Unchecked, excessive ROS can lead to nucleic acid, lipid, and protein damage and ultimately cell death via apoptosis or necrosis.

Given their high metabolic rate, limited regeneration potential, and relatively low baseline levels of antioxidants, neurons are thought to be particularly vulnerable to ROS. Studies of human postmortem brain tissue have repeatedly implicated oxidative stress in neuronal death, particularly in later-onset neurodegenerative disorders. For example, DNA and protein oxidation, and lipid peroxidation are observed in the substantia nigra of patients with Parkinson's disease (PD) (Jenner and Olanow, 1996). The increase in ROS correlates with multiple findings of mitochondrial complex 1 deficiencies in PD patients, studies promoted by the finding that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), paraquat, and rotenone compounds known to inhibit complex 1, also induce PD pathophysiology (Parker and Swerdlow, 1998; Dawson and Dawson, 2003). Similarly, increases in various biomarkers of oxidative stress have also been observed in diseased regions of postmortem brain tissue from patients with Alzheimer's disease, amyotrophic lateral sclerosis, prion disorders, Huntington's disease, and progressive supranuclear palsy (Andersen, 2004; Barnham et al., 2004).

Because cell cycle misregulation, like oxidative stress, is associated with human late-onset neurodegenerative disorders, the suggestion arises that these processes may be molecularly intertwined. At first, this idea of a connection seems to run counter to the many reports that ROS-induced DNA damage correlates with cell cycle arrest, not reentry. The effects of ROS on cell cycle progression, however, appear to be dose dependent. For example, whereas high doses of H_2O_2 induce cell cycle arrest and cell death, low doses stimulate proliferation or survival. This suggests that below toxic concentrations, H_2O_2 , like nitric oxide, can serve as a signaling molecule (D'Souza et al., 1993; Burdon, 1995; Burdon et al., 1996; Herbert et al., 1996; Ohguro et al., 1999; Clerk, 2003). A better example is the *harlequin* (*Hq*) mutant mouse.

Harlequin is an X-linked mutation; *Hq/Hq* and *Hq/Y* mice develop progressive ataxia associated with the death of both granule and Purkinje cells in cerebellum, as well as ganglion, amacrine, horizontal, and photoreceptor cells of the retina (Klein et al., 2002). The *Hq* mutation is caused by a proviral insertion in the first intron of the *apoptosis-inducing factor* (*Aif*) gene, result-

ing in an 80–90% reduction of both transcripts and protein. *Aif* is a ubiquitously expressed flavoprotein with structural homology to glutathione reductase, an important recycling component of the glutathione H_2O_2 -scavenging system (Susin et al., 1999; Maté et al., 2002). Downregulation of *Aif* in *Hq* mutant mice is associated with increases in oxidative damage: lipid peroxidation, catalase, and total glutathione. Significantly, these increases are observed months before the onset of neurodegeneration. In keeping with these findings, mutant cerebellar granule cells are more sensitive to H_2O_2 -mediated cell death, a sensitivity that is rescued by expression of full-length *Aif* cDNA. Furthermore, there is an increase in the levels of DNA oxidation, as measured by the presence of 8-hydroxyguanine (8-OHdG), a DNA lesion resulting from unrepaired oxidative damage. Together, these studies suggest that *Aif* regulates the amount of ROS in cerebellar and retinal neurons by unidentified mechanisms (Lipton and Bossy-Wetzel, 2002; Bonni, 2003).

In addition to the changes in oxidation damage repair, the neurons in the *Hq* mouse also suffer from a failure of cell cycle control. PCNA (proliferating cell nuclear antigen) and CDC47 (cell division cycle 47) immunostaining are found in susceptible *Hq* neurons as is BrdU-labeling, demonstrating that *Hq* retinal neurons reenter a cell cycle (Klein et al., 2002). The number of granule cells demonstrating cell cycle markers increases with age, peaking at 7 months when most cell death occurs. That cell cycle reentry is tightly associated with cell death in these cells is indicated by the finding that all apoptotic neurons express S-phase markers. Furthermore, all neurons that had re-entered into S-phase had oxidized DNA, as evidenced by their immunoreactivity with antibodies to 8-OHdG. Not all 8-OHdG-positive neurons, however, were cell cycle positive, suggesting that the oxidative damage precedes cell cycle reentry.

The mechanisms by which oxidative stress induces cell cycle abnormalities in postmitotic neurons is not known (Klein and Ackerman, 2003). ROS might alter the components of a mitogenic signaling pathway by ectopically activating either a growth factor receptor (Holbrook and Ikeyama, 2002) or its signaling molecules such MAP (mitogen-activated protein) kinase and NF- κ B (nuclear factor κ B) that are farther downstream (Mardale and Holbrook, 2002). Another intriguing possibility is that the ROS-induced DNA damage itself might initiate cell cycle reentry in postmitotic neurons. The relative timing of the appearance of oxidized DNA (8-OHdG immunoreactivity) and cell cycle events (BrdU, PCNA, etc.) in *Hq* neurons is one piece of evidence, but there are others. Abortive cell cycle reentry and subsequent apoptosis is known to occur in cultured postmitotic neurons treated with compounds that induce chromosomal breaks and base mis-incorporation (Kruaman et al., 2004). Also, DNA repair proteins have been shown to physically interact with proteins involved in DNA replication (Nagelhus et al., 1997; Otterlei et al., 1999), further suggesting a linkage. Finally, expression and/or activity of several DNA repair enzymes appear to be regulated by the cell cycle and are highest in proliferating cells (Nilsen et al., 2000; Choi et al., 2002). Perhaps the DNA strand breaks and oxidized DNA bases that have been observed in postmortem tissue from patients with neurodegenerative disorders (Cotman and Su, 1996; Davydov et al., 2003) are not efficiently repaired. If not, it may be that they introduce mutations and thus interfere with neuronal transcription and other DNA-dependent processes. If the damage is too great, a critical component of the cell cycle control pathway might be lost in cells, leading to apoptosis: divide and die.

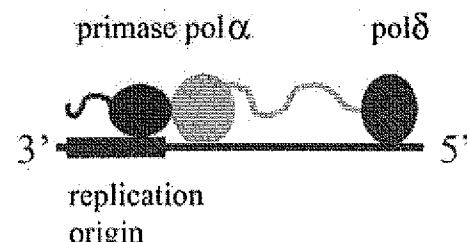
What is the nature of the cell cycle events observed in Alzheimer's disease?

This naturally returns the discussion to the question is just what is a cell cycle in a nondividing cell besides a classic oxymoron. The studies of A β carry a clear message: there are more ways than one to engage a neuronal cell cycle, but whenever this happens the net effect is usually bad. The examples of APP signaling and oxidative damage are but two examples (and the A β peptide itself is another). However, if all of these stimuli lead to the same common pathway, then surely we would be well advised to learn as much as we can about the events that are triggered. With the war on cancer now nearly 35 years old, the depth of our knowledge of the normal mechanics of the eukaryotic cell cycle is considerable. Classically, the reentry of a mammalian cell into the cell cycle depends on extracellular proliferative signals, which exert their effect by the sequential activation of CDK (cyclin-dependent kinase) complexes (for review, see Grana and Reddy, 1995). The cyclin D/CDK4–6 complexes control the transition of cells from G₀ to G₁-phase. The G₁/S transition and the S-phase progression are regulated in part by the activation of the cyclin E/CDK2 complex and of the cyclin A/CDK2 complex, respectively. The cyclin B/CDK1 complex is the regulator of the G₂/M transition. After an appropriate stimulus to a differentiated neuron (the A β peptide, for example), there is a rise in cyclin D1 and an increase in the levels of phosphorylation of the retinoblastoma protein. This is followed by the induction of cyclins E and A. The treated neurons start DNA replication but then appear to undergo apoptosis instead of completing a full mitotic cycle (Copani et al., 1999). This abortive attempt at cell division has true biological meaning, however, because analysis by fluorescent-activated cell-sorting techniques has shown that blockade of the G₁/S transition using a cyclin D1 antisense or a dominant-negative mutant of CDK2 prevented both A β -induced DNA replication and apoptosis (Copani et al., 1999). These experiments, as well as the studies cited above, show that highly differentiated neurons can be induced to express significant amounts of cell cycle proteins (e.g., cyclins) and enter a process of DNA replication that leads to death and not division (Freeman et al., 1994; Park et al., 1997; Giovanni et al., 1999). The reactivation of the cell cycle is an obligatory step in this pathway (not just a by product) because blocking the cycle blocks the death. The question that remains is how does the ectopic S-phase trigger the death of neurons. This understanding requires a more detailed analysis of the biochemistry of eukaryotic DNA replication.

The DNA replication machinery is activated by A β in adult neurons

In proliferating cells, replication involves three distinct DNA polymerases (pol δ), pol α , pol δ , and pol ϵ (for review, see Hubbscher et al., 2000). Pol α , with its associated primase activity, is unique among DNA pols because of its ability to initiate *de novo* DNA replication by synthesizing short RNA-DNA primers. These are extended by either pol δ or pol ϵ , which are endowed with a proofreading exonuclease activity that is suited to minimizing errors during replication. The complete pol α consists of four polypeptides with molecular mass of 180, 70, 58, and 49 kDa. The p180 subunit carries the DNA polymerase activity, whereas the p49 subunit harbors the catalytic primase activity. Pol α helps to hold the cell cycle in check (for review, see Arezi and Kuchta, 2000), whereas pol δ and pol ϵ can function as DNA repair enzymes, correcting any mismatch errors that have escaped the editing process during replication. Thus, the coordinated regula-

Canonical DNA replication in dividing cells



Non-Canonical DNA replication in neurons

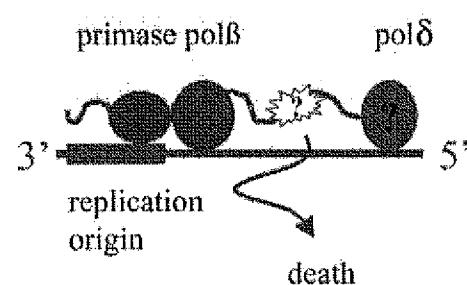


Figure 2. A noncanonical pathway of DNA replication is mediated by pol β and is causally related to neuronal death. In dividing cells (top), DNA replication (yellow strand) is accomplished by DNA pol α and pol δ/ϵ , which follow one another in the elongation of the short RNA primer synthesized by the primase (red). In neurons challenged with A β (bottom), DNA replication (purple strand) is performed by pol β that substitutes for pol α . The error-prone activity of pol β might generate DNA damage, eventually leading to cell death. Some DNA replication is possibly mediated by pol δ but does not contribute to neuronal death (Copani et al., 2002).

tion of the activity of multiple DNA polymerases is a fundamental aspect of DNA replication in normally dividing cells.

There are other DNA polymerases that are specialized to specifically ignore DNA damage during replication. The tradeoff is that these enzymes introduce errors at a high frequency when they operate on undamaged DNA (Friedberg et al., 2002). One of these, pol β , participates in base excision repair, a pathway that is used to replace any oxidized bases that may appear over time (Sobol et al., 1996). The precise repertoire of DNA polymerases that are expressed by neurons is unknown. Most of our current information comes from studies of the differential sensitivity of the polymerases to different inhibitors. In isolated neuronal fractions, pol β activity tends to predominate, although some pol δ/ϵ and a little pol α activity are also present (Raji et al., 2002).

In dissociated culture, it appears that neurons constitutively express pol δ and its ancillary protein PCNA, whereas pol ϵ is undetectable (Copani et al., 2002). After exposure to A β , neurons express the p49 and the p58 subunits of the primase/pol α complex, whereas the expression of replicative polymerases (pol α , pol δ , and pol ϵ) remains unaffected. Unexpectedly, the repair enzyme pol β is induced in response to A β in a cell cycle-dependent manner that is unusual in proliferating cells. This increase in protein appears to have functional consequences because the knockdown of pol β prevented A β -induced DNA synthesis and the ensuing apoptosis. Similar effects are observed by knocking down the p49 primase subunit. These results suggest the existence of a primase-directed noncanonical pathway of DNA replication, which is mediated by pol β in A β -treated neurons (Fig. 2).

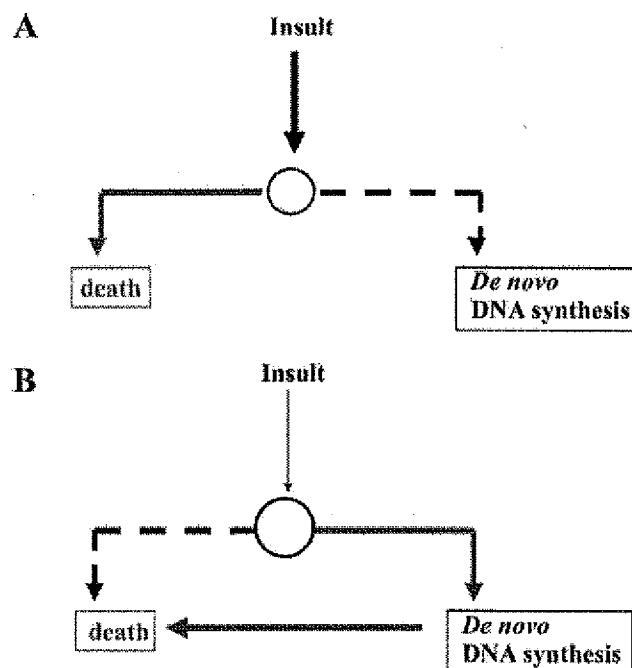


Figure 3. The involvement of DNA replication in neuronal death depends on the type of insult and the susceptibility of neurons. *A*, A susceptible neuron (smaller circle) in the presence of a severe insult (thick vertical arrow), which possibly causes extensive DNA damage, dies without the contribution of *de novo* DNA synthesis. *B*, In a less vulnerable neuron (bigger circle) exposed to other types of insults, including $\text{A}\beta$ or mild oxidative stress, *de novo* DNA synthesis becomes essential for neuronal death.

These results are as important as they are unexpected. Nonetheless, they leave unanswered the question of how this unorthodox pathway of DNA replication leads to neuronal apoptosis. Given that $\text{pol}\beta$ is an error-prone enzyme, it is possible that its use contributes to a level of DNA damage that proves toxic to the neuron. Such damage is reported in AD neurons (Su et al., 1997), along with an increased expression of p53, a sensor of DNA damage (de la Monte et al., 1997). When $\text{pol}\beta$ induction is prevented, the increase in p53 is not seen (Copani et al., 2002). The presence of hyperploid neurons in AD brain (Yang et al., 2001) suggests that this error-prone replication mechanism somehow proceeds without signaling to cell cycle checkpoints. If this entire process were driven by $\text{pol}\beta$, the prediction would be that the abnormal neurons are loaded with unsustainable amounts of DNA damage. In other words, *de novo* DNA synthesis might be a potential source of replication errors that are sufficient to trigger the activation of a p53-dependent death pathway in neurons. This process need not be all-or-none; the outcome might depend on the severity of the insult and/or on the vulnerability of the neurons (for examples of the different phenotypes of neuronal death in the harlequin mutant mice, see Klein and Ackerman, 2003) (Fig. 3).

Conclusions and cautions

Cell cycling, it seems, is incompatible with the complex differentiated state of a neuron. Forcing the former in the presence of the latter results in neuronal death by an as yet unknown mechanism. In other words, if you are a neuron, “you divide, you die.” In an era of information overload, one might wish to avoid the implications of this deceptively simple relationship, but evidence is mounting that there is new biology under the surface. Although there are significant technical hurdles to overcome in the study of cell cycle regulation in adult neurons, these difficulties must be

addressed. This is true in part because of the growing number of situations in which the behavior of nerve cells is governed by the divide-and-die dictum. In neurodegenerative disease as well as in normal development, we are becoming aware that the state of the genome is not always static. Major genomic changes involving extensive amounts of new DNA synthesis can occur. These changes are visible either by monitoring the incorporation of BrdU or through the direct query of the genome using FISH techniques. That genome-level changes occur is certain. It is the nature of these events that is unclear at present.

The questions that remain are large but clear. What is the state of the genome in a CNS neuron? The prohibition against cell division can be overcome in most other cells (hence, the large number of different types of cancer), but there are hints that things are not straightforward in the brain (Geller and Potter, 1999; Rehen et al., 2001; Kaushal et al., 2003; A. H. Yang et al., 2003). So what is different about a nerve cell? What is it about the reengagement of a cell cycle process that kills a neuron? It would help the discovery process if we could determine whether the events described in this article represent a true mitotic cell cycle or something different. Unraveling the biochemistry and cell biology of the process we have called a neuronal cell cycle is an important first step. Along the way, we need to discover whether the decision to divide is destructive (as suggested by studies such as the T-antigen transgenics) or protective (as hinted at by the apparent long survival of the hyperploid neurons in mouse and man). Other questions have importance for therapeutic intervention in specific disease situations. What drives a neuron to divide? Is it activity, heightened trophic factor levels, other cytokines, or something novel? What are the signaling pathways that relay this impulse to the cell “division” machinery?

Cell cycling is as old as life itself. In most situations, it is a generative force that creates new cells from old. However, in the brain, there are persistent associations between cell cycling and the loss of neurons to cell death. This is a paradox to be sure, but, in a field that is known for its surprises, we should probably expect nothing less.

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